Molecular identification and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs in Mexico

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ABSTRACT

The tick-borne pathogens *Ehrlichia canis* and *Anaplasma platys* are the causative agents of canine monocytic ehrlichiosis (CME) and canine cyclic thrombocytopenia (CCT). Although molecular evidence of *E. canis* has been shown, phylogenetic analysis of this pathogen has not been performed and *A. platys* has not been identified in Mexico, where the tick vector *Rhipicephalus sanguineus* sensu lato (s.l.) is common. The aim of this research was to screen, identify and characterize *E. canis* and *A. platys* by PCR and phylogenetic analysis in dogs from La Comarca Lagunera, a region formed by three municipalities, Torreon, Gomez-Palacio and Lerdo, in the Northern states of Coahuila and Durango, Mexico. Blood samples and five engorged *R. sanguineus* s.l. ticks per animal were collected from 43 females and 57 male dogs presented to veterinary clinics or lived in the dog shelter from La Comarca Lagunera. All the sampled dogs were apparently healthy and PCR for *Anaplasma 16S rRNA*, *Ehrlichia 16S rRNA*, and *E. canis trp36* were performed. PCR products were sequenced and used for phylogenetic analysis. PCR products were successfully amplified in 31% of the samples using primers for *Anaplasma 16S rRNA*, while 10% and 4% amplified products using primers for *Ehrlichia 16S rRNA* and *E. canis trp36* respectively. Subsequent sequencing and phylogenetic analyses of these products showed that three samples corresponded to *A. platys* and four to *E. canis*. Based on the analysis of *trp36* we confirmed that the *E. canis* strains isolated from Mexico belong to a conservative clade of *E. canis* and are closely related to strains from USA. In conclusion, this is the first molecular identification of *A. platys* and the first molecular characterization and phylogenetic study of both *A. platys* and *E. canis* in dogs in Mexico.

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1. Introduction

The genera *Anaplasma* and *Ehrlichia* belong to the family Anaplasmataceae, which includes intracellular Gram-negative organisms that multiply within membrane-bound vacuoles (de la Fuente et al., 2006; Gaff et al., 2014). These organisms are transmitted by ixodid ticks to vertebrate hosts such as humans, domestic and wild animals (Gaunt et al., 2010; Gaff et al., 2014).

Anaplasma platys, previously known as *Ehrlichia platys*, infects platelets and is the causative agent of canine cyclic thrombocytopenia (CCT) in dogs (Gaff et al., 2014), and has also been identified in cats (Lima et al., 2010), humans (Maggi et al., 2013; Breitschwerdt et al., 2014), and foxes (Cardoso et al., 2015). *A. platys* was first described in the United States (Harvey et al., 1978). Since then, it has been widely reported in Greece (Kontos et al., 1991), Spain (Sainz et al., 1999), Japan (Inokuma et al., 2001; Motoi et al., 2001), Australia (Brown et al., 2001), France (Beaufils et al., 2002), Venezuela (Arraga-Alvarado et al., 2003; Huang et al., 2005), Italy (de la Fuente et al., 2006), Thailand (Pinyoowong et al., 2008), Israel (Harrus et al., 2011), and Algeria (Dahmani et al., 2015). The vector of *A. platys* is probably *R. sanguineus* s.l., but so far, the role of this tick in the transmission of *A. platys* is unclear (Dahmani et al., 2015).

Ehrlichia canis infects mononuclear cells causing a disease known as canine monocytic ehrlichiosis (CME) in dogs, wolves and jackals (Gaff et al., 2014), and a closely related organism was suggested to infect humans in Venezuela, although this

have not been confirmed (Perez et al., 2006). Clinically, animals may show fever, lethargy, apathy, anorexia, pale mucous membranes, splenomegaly, hemorrhages and uveitis (Nakaghi et al., 2008), but dogs may remain subclinical persistent carriers for prolonged periods (Gaunt et al., 2010). *E. canis* was first described in Algeria (Donatien and Lestoquard, 1935) and has been widely identified in several countries in America, (Aguiar et al., 2007, 2013), Europe (Cardoso et al., 2012), Asia (Pinyoowong et al., 2008), and Africa (Dahmani et al., 2015). Molecular identification of *E. canis*, has been based on the identification of the *16S rRNA* (Vinasco et al., 2007; Pinyoowong et al., 2008; López et al., 2012) or the *trp36* gene (Aguiar et al., 2013; Zweygarth et al., 2014). *E. canis* is transmitted by the brown dog tick *R. sanguineus* s.l. (Nava et al., 2015; Gaff et al., 2014) transstadially, when the pathogen is acquired by an immature phase and transmitted by a subsequent developmental phase; and intrastadially, when the same tick stage acquires and transmits the pathogen (Stich et al., 2008).

In Mexico, a serologic study of *E. canis* reported a prevalence of 33.1% (Nuñez-Ochoa, 2003), while in the state of Yucatan a prevalence of 44.1% was found (Rodríguez-Vivas et al., 2005), and more recently, a prevalence of 74.3% was found in the state of Sinaloa (Sosa-Gutierrez et al., 2013). In spite of serological and molecular evidence of *E. canis* (Pat-Nah et al., 2015) in Mexico, phylogenetic analyses of *E. canis* have not been performed, and so far *A. platys* has not been identified either serologically or molecularly. Therefore, the aim of this research was to screen, identify and characterize *A. platys* and *E. canis* by PCR and phylogenetic analysis of the genes *16S rRNA*, and *trp36*, in dogs from La Comarca Lagunera Mexico, an endemic region of the tick vector *R. sanguineus* s.l.

2. Material and methods

2.1. Ethics statement

Sampling was conducted complying with the current Mexican Legislation of Animal Health (LFSA, 2007), and designed and developed in accordance with the protocols established by the Committee for Care and Use of Animals at the National University of Mexico (CICUA, 2014).

2.2. Study site

The present study was carried out in "La Comarca Lagunera", a region located in the western part of the Chihuahuan Desert (north-central Mexico) in the states of Coahuila and Durango, Mexico (Fig. 1) and is formed by a semi-desert plain at an altitude between 1000 and 1200 m above sea level (Czaja et al., 2014). The region is located between the latitude 25°15′ N–25°40′ N and longitude 102°55′ O–103°50′ O, the area extension is of 4900 km^2. The average annual temperature is 21 °C, and 100–400 mm of precipitation, with a semi-desert climate and low atmospheric humidity (INEGI, 2009; SMN-CNA, 2011).

Fig. 1. Map of the study area. The black area represents *La Comarca Lagunera* formed by the municipalities of Torreón, Coahuila (1), Gómez Palacio, and Lerdo, Durango (2, 3).